Seizure 24 (2015) 124–126

Contents lists available at ScienceDirect

Seizure

journal homepage: www.elsevier.com/locate/yseiz

Short communication

Polymorphism of the multidrug resistance 1 gene MDR1/ABCB1 C3435T and response to antiepileptic drug treatment in temporal lobe epilepsy

Ida Manna [a], Antonio Gambardella [b,*], Angelo Labate [b], Laura Mumoli [b], Edoardo Ferlazzo [c], Franco Pucci [b], Umberto Aguglia [b,c], Aldo Quattrone [a,b]

[a] Institute of Molecular Bioimaging and Physiology (IBFM), Section of Germaneto, National Research Council, Catanzaro, Italy
[b] Institute of Neurology, University "Magna Graecia", Germaneto, Catanzaro, Italy
[c] Regional Epilepsy Centre, Hospital of Reggio Calabria, Reggio Calabria, Italy

ARTICLE INFO

Article history:
Received 17 March 2014
Received in revised form 22 September 2014
Accepted 25 September 2014

Keywords:
MDR1
Pharmacogenetics
Antiepileptic drugs
Association studies
Single nucleotide polymorphism
Temporal lobe epilepsy

ABSTRACT

Purpose: A synonymous C to T variant at position 3435 (c.3435C>T) is one common polymorphism of the multidrug resistant 1 (MDR1) gene, which encodes the major transmembrane efflux transporter P-glycoprotein. It has been suggested that this polymorphism, and more specifically the 3435CC genotype, may be associated with the response to antiepileptic drug treatment. Here we wished to examine the role of such a candidate variant in a cohort of 175 patients (98 women and 76 men; mean ± SD age: 47.90 ± 17.64) with temporal lobe epilepsy (TLE).
Methods: Patients were classified according to whether they had drug-responsive ($n = 134$) or drug-resistant ($n = 41$) epilepsy. We also enrolled 175 healthy controls (93 women and 82 men; mean ± SD age: 72.5 ± 6.8), matched for sex and ethnicity.
Results: Patients and controls were genotyped for detection of the 3435C>T polymorphism, but the analysis showed no significant association between the CC genotype and the risk of drug-resistant epilepsy.
Conclusion: These findings rule out the MDR1 c.3435C>T polymorphism having a major role or increasing the risk of drug-resistance suggesting a revision is required to determine the contribution of this polymorphism in predicting drug response in epilepsy.

1. Introduction

Epilepsy is one of the most prevalent chronic neurological disorders, affecting approximately 1–2% of the population.[1] About one third of patients with epilepsy have poor seizure control and experience recurrent seizure events despite appropriate therapy with antiepileptic drugs (AEDs).[2] The mechanisms underlying the resistance to AEDs in the epilepsy treatment are still not well-understood, although efforts to predict pharmacoresistance have revealed several risk factors such as an early onset of epilepsy, etiology, type of epilepsy, and environmental factors.[3] Moreover, genetic variants are supposed to play an important role in the development of pharmacoresistance in patients with epilepsy.

Growing evidence would indicate that an increase of functional expression of multidrug transporters produces pharmacokinetic changes that modify the access of AEDs to central nervous system targets.[4] Genetic polymorphisms in these transporters could account for their increased expression and/or functional activity.[5] P-glycoprotein (P-gp) is the most studied protein among the ATP-binding cassette (ABC) efflux transporters. The ABC subfamily B member 1 transporter (ABCB1) gene, also known as multidrug resistance 1 (MDR1), encodes this transmembrane transporter. In recent years, according to the single nucleotide polymorphism (SNP) database maintained by the National Center for Biotechnology Information (NCBI), more than 50 SNPs have been identified that may alter the expression of P-gp or its protective physiological function. A silent mutation in the MDR1gene, c. 3435C>T

* Corresponding author at: Cattedra ed U.O. di Neurologia, Università degli Studi "Magna Graecia" Catanzaro, Campus Universitario di Germaneto, 88100 Catanzaro, Italy. Tel.: +39 0961 3647270; fax: +39 0961 3697177.
E-mail address: a.gambardella@unicz.it (A. Gambardella).

http://dx.doi.org/10.1016/j.seizure.2014.09.010
1059-1311/

(rs1045642), in exon 26 may decrease the levels of mRNA and protein or alter the protein structure and substrate affinity.[6] Several studies have focused on this common SNP to explore the association with disease development and drag response in epilepsy but none have been conclusive.[7,8]

In the light of these controversial findings, the aim of the present study was to investigate whether c.3435C>T polymorphism is more prevalent in patients with epilepsy versus control subjects, and whether this gene may serve as a biomarker for pharmacoresistance in TLE.

2. Materials and methods

2.1. Patients

A total of 175 patients (98 women and 76 men; mean ± SD age: 47.90 ± 17.64) with temporal lobe epilepsy (TLE) were included in this study. Data and evaluation procedures on our TLE patients have been reported in greater detail elsewhere.[9–10] All patients received a diagnosis of non-lesional TLE, based on comprehensive clinical, neuropsychological, electroencephalographic, and brain MRI evaluations. The diagnosis of TLE was mainly based on typical temporal auras and/or interictal EEG discharges with a maximum over the temporal lobes. In all patients, brain MR images were obtained using sequences and slices to optimize visual detection of mesial temporal structures. Based on the MR study, the TLE was classified as non-lesional if no focal mass lesions such as cerebral tumors, cortical dysgenesis, vascular lesions or malformations, or post-traumatic scars were detected. TLE patients with neuroimaging evidence of mesial temporal sclerosis were included. Brain MRI study revealed evidence of hippocampal sclerosis (Hs) in 32/175 (18%) patients.

In all patients, the most utilized AEDs were carbamazepine, oxcarbazepine, lamotrigine or topiramate as monotherapy or in combination. According to the International League against Epilepsy,[11] drug-resistance was defined as the failure of adequate trials of two tolerated, appropriately chosen and administered AEDs (whether as monotherapy or in combination) to achieve seizure freedom. Patients were classified according to whether they had drug-responsive ($n = 134$) or drug-resistant ($n = 41$) epilepsy. Neurological examinations were unremarkable in all patients. None of our patients had mental retardation.

We also enrolled 175 healthy controls (93 women and 82 men; mean ± SD age: 72.5 ± 6.8), matched for sex and ethnicity. Patients and controls were genotyped for detection of the c.3435C>T polymorphism using TaqMan Allelic Discrimination assays, on an Applied Biosystems PCR platform. All patients and controls were Caucasian and were born in Italy. All tested individuals signed the informed consent for the genetic testing.

2.2. Genotyping and mutation analysis

Genomic DNA was extracted from the whole blood using Wizard Genomic DNA purification kit according to the instructions of the supplier (Promega, Madison, WI). Samples were genotyped for the c.3435C>T (rs1045642) polymorphism (GeneBank: NM_000927.4) by TaqMan based allelic discrimination assay (Applied Biosystems, Foster City, CA, USA). Assay conditions were in accordance with manufacturers' protocols. Fluorescence outputs were quantified in real time by using a 7900HT Real Time PCR System, and the data were analyzed using SDS software, version 2.2.2 (Applied Biosystems).

2.3. Statistical analysis

Allelic and genotype frequencies were estimated by direct counting. A Chi square (χ^2) test was used to evaluate for deviation of genotype frequencies from Hardy–Weinberg equilibrium. The frequency of alleles and genotypes between patients and controls were compared using χ^2 test. Starting from contingency tables constructed were calculated Odds Ratios (ORs) and their 95% confidence intervals (CIs). To test the hypothesis of equality of means between the ages of drug-resistant and drug-responsive patients and controls we used the one-way analysis of variance, followed by Student's *t*-test for multiple comparisons, correcting the resulting *p*-value according to the Bonferroni method. Odds ratios and Confidence intervals were calculated according to a multivariate logistic regression model adjusted for age and sex. Statistical analysis was performed with Statistical Package for Social Science software (SPSS, version 12.0, Chicago, IL, USA) for Windows.

3. Results

No significant deviations from Hardy–Weinberg equilibrium were observed in epilepsy patients ($p = 0.93$). Moreover, our sample gave sufficient statistical power (>0.80) to detect the difference at the 0.05 (two-tailed) level. Table 1 shows the genotype and allele frequencies of the polymorphisms in the MDR1 gene for both drug-resistant and drug-responsive epilepsy patients. The distribution of MDR1 c.3435 C>T genotypes within drug-resistant and drug-responsive groups were not significantly different from that under Hardy–Weinberg equilibrium ($p = 0.76$ and $p = 0.79$, respectively). No statistically significant differences were detected for the genotype and allele frequencies of the polymorphisms in the MDR1 gene between the drug-resistant and drug-responsive groups ($p > 0.05$). Moreover, there was non-significant odds ratio (Table 1), indicating that response to treatment was not affected by the presence or absence of the MDR1 polymorphism.

Table 1
Genotypic and allelic distributions of the MDR1 c.3435C>T polymorphism in relation to AED response.

c.3435C>T polymorphism	AED responsive ($n = 134$)	AED resistant ($n = 41$)	CTRLs ($n = 175$)	OR 95% CI (responsive vs CTRL)	OR 95% CI (resistant vs CTRL)	*p*-Value
Genotype						
CC	45 (33.6%)	13 (31.7%)	48 (27.4%)	1.0	1.0	0.705
CT	64 (47.8%)	21 (51.2%)	98 (56.0%)	0.697 (0.416–1.165)	0.791 (0.365–1.714)	
TT	25 (18.7%)	7 (17.1%)	29 (16.6%)	0.920 (0.470–1.801)	0.891 (0.319–2.491)	
c.3435C>T polymorphism	AED responsive ($n = 268$)	AED resistant ($n = 82$)	CTRLs ($n = 350$)	OR 95% CI (responsive vs CTRL)	OR 95% CI (resistant vs CTRL)	*p*-Value
Allele						
C	154 (57.5%)	47 (57.3%)	194 (55.4%)	1.0	1.0	0.867
T	114 (42.5%)	35 (42.7%)	156 (44.6%)	0.921 (0.6681.269)	0.926 (0.570–1.505)	

Table 2
Studies of MDR1 c.3435C>T polymorphism in relation to drug-resistance among epilepsy patients and controls based on the ethnicity.

Studies	Country	Ethnicity	Drug-resistant (N of patients)	Drug-responsive (N. of patients)	Control (N of subjects)	Results
Chen L et al. (2007)	China	Asian	50	164	–	Negative
Wang SY et al. (2008)	China	Asian	40	40	40	Negative
Gao X et al. (2009)	China	Asian	70	62	62	Negative
Von Stulpnagel C et al. (2009)	Germany	Caucasian	160	71	319	Negative
Alpman A et al. (2010)	Turkey	Caucasian	38	–	87	Negative
Dong L et al. (2011)	China	Asian	157	193	368	Negative
Emich-Widera E et al. (2013)	Poland	Caucasian	60	25	100	Negative
Saygi S et al. (2014)	Turkey	Caucasian	59	60	76	Negative

4. Discussion

The mechanisms of drug resistance in epilepsy have not been established. Various genetic polymorphisms in the MDR1 gene could be linked with altered *in vivo* transport activity of P-gp, which could be a facilitator in drug-resistant epilepsy.[5] It has been speculated that the common SNP c.3435C>T in the MDR1 gene, specifically the 3435CC genotype, is associated with drug resistance to anticonvulsants.[8] Although several association studies on the MDR1 gene with drug disposition and disease susceptibility have been completed to date, the data remain unclear and incongruous. Therefore, in the present study, we investigated the common SNP c.3435C>T in the MDR1 gene in drug-resistant and drug-responsive epilepsy patients. Results of the present study demonstrated that genotype and allele frequencies of c.3435C>T polymorphisms of the MDR1 gene did not differ between drug-responsive and drug resistant epilepsy patients. The results from various studies focusing on the role of MDR1 c.3435C>T polymorphism in AED responsiveness are conflicting.[7,8] Consistent with the present study, several studies have found no significant associations between drug-resistance epilepsy and the MDR1 c. 3435C>T polymorphism (Table 2). The differences observed in these reports may be due to the ethnic differences in genotype/allele frequencies, because genetic polymorphisms often vary between ethnic groups.

In conclusion, we failed to show an association between the c.3435C>T polymorphism and the risk of drug-resistant epilepsy in the study population. The limitation of the current study is the small sample size. So, our results cannot definitively rule out a role for this gene, although derived from a small but ethnically homogeneous sample size, but provides further evidence that although the MRD1 gene has been highly investigated and plays many important roles, it is unlikely that the polymorphism c.3435C>T plays a major role in the development of epilepsy or in drug resistance to anticonvulsants. Despite the biological plausibility, several studies, including our own, have now failed to confirm a relationship between the c.3435C>T polymorphism and drug resistance. The limited contribution of a SNP should also be taken into account. Because the inherited component of the response to drugs is typically polygenic,[12] the impact of a single gene may be confounded by influences from other genes and by environmental factors. It remains to be determined whether haplotypes in the MDR1 gene play a role in epilepsy susceptibility or in the risk of drug-resistance.

Conflict of interest statement

None declared.

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